Global Microbial Threats

Emerging Resistance in Clinically Important Gram-Positive Cocci

CLYDE THORNSBERRY, PhD, *Franklin, Tennessee*

In the first half of the decade of the 1990s, we in the United States have seen the emergence and escalation of substantial antimicrobial resistance in medically important gram-positive cocci. The incidence of methicillin resistance of *Staphylococcus aureus* continues to increase (now 18%), resulting in many more isolates that are multiply resistant; all *S aureus* isolates are still susceptible to vancomycin. Enterococci, particularly *Enterococcus faecium*, have increasingly developed resistance to penicillin, gentamicin, streptomycin, and vancomycin (the last plasmid-mediated). More than a fourth of *Streptococcus pneumoniae* strains are now resistant to penicillin, and these strains tend to be multiply resistant, including to cephalosporins and macrolides.

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Many species of gram-positive cocci were important pathogens four to five decades ago, but then took a back seat to gram-negative pathogens. Gram-positive cocci have now returned with a vengeance, and these have a greater resistance to many of the antimicrobial agents.

In the 1950s and into the 1960s, the scourge of hospitals in the United States and the rest of the world was *Staphylococcus aureus*. Because penicillin had antistaphylococcal activity, its major use was to treat staphylococcal infections. Staphylococci soon acquired the ability to produce a β-lactamase, however, and became resistant to penicillin. Consequently, the penicillinase-resistant penicillins were developed and introduced in the early 1960s. With the introduction of these newer penicillins and their ability to inhibit or kill β-lactamase–producing staphylococci, gram-positive organisms became less of a problem, and gram-negative organisms became the major nosocomial pathogens. In the latter part of the 1960s through the early 1980s, the leading nosocomial pathogens were gram-negative. In fact, *Escherichia coli* is still the most frequent nosocomial pathogen in the National Nosocomial Infections Surveillance studies (about 100 institutions), and *Pseudomonas aeruginosa* is one of the five most frequent species (Table 1).[1] In recent years, however, gram-positive organisms have reemerged as important nosocomial pathogens. Thus, any developing or increasing resistance to antimicrobial agents that are normally used to treat gram-positive organisms is medically important.

In most staphylococci, and particularly *S aureus*, the incidence of β-lactamase production had developed to a point where essentially all staphylococci were considered, from a clinical point of view, to be resistant to penicillin. Drugs such as methicillin, however, introduced during the early 1960s, were active against the strains that produced penicillinase and, thus, were the major agents for the treatment of staphylococcal infections. Almost as soon as methicillin was introduced to medical use, methicillin-resistant staphylococci were identified and actually continued to be identified in the 1960s and 1970s.[2] In fact, in the 1960s, methicillin-resistant *S aureus* was a major nosocomial pathogen in Europe,[3] even though relatively rare in the United States. Beginning in the 1970s, the number of strains of methicillin-resistant *S aureus* began to increase and has continued to do so.

Penicillin-resistant pneumococci have also been known since the 1960s, but even into the 1980s, the number of penicillin-resistant pneumococci was fairly low in the United States. Although enterococci have always been recognized as bacteria that are innately "relatively resistant" to a large number of antimicrobial agents, up until recent times, they were assumed to be susceptible to certain drugs, such as ampicillin, which was used for therapy for less severe enterococcal infections. They were also assumed to be susceptible to vancomycin and combinations of a penicillin and aminoglycoside, which were used for more severe enterococcal infections.

Resistance in Staphylococci

As early as the mid-1940s, it was shown that a clinical isolate of *S aureus* could produce an enzyme that would inactivate the penicillin drug and confer penicillin resistance to that particular organism.[4] At that time, the enzyme was not called a β-lactamase but is in fact the first description of β-lactamase (or penicillinase) in *S*

From the MRL Pharmaceutical Services, Franklin, Tennessee.
Reprint requests to Clyde Thornsberry, PhD, MRL Pharmaceutical Services, 357 Riverside Dr, Franklin, TN 37064.

ABBREVIATIONS USED IN TEXT

MIC = minimal inhibitory concentration
NCCLS = National Committee for Clinical Laboratory Standards

aureus. During the late 1940s and 1950s, the number of strains of staphylococci that produced β-lactamase increased to such a level that penicillin-resistant *S aureus* became a definite clinical problem. Indeed, in our latest national surveillance study, 93% of *S aureus* strains identified in the United States produced β-lactamase and were resistant to penicillin (unpublished data). Because of these high rates of β-lactamase production, all *S aureus* strains are considered resistant to penicillin.

In the early 1960s, the first of the penicillinase-resistant penicillins, methicillin, was introduced.[2] This drug was active against all *S aureus* strains, including those that produced β-lactamase and were resistant to penicillin. Other penicillinase-resistant penicillins were soon introduced, such as cloxacillin, nafcillin, and oxacillin. Although penicillinase-resistant penicillins remained major drugs for the treatment of *S aureus* infections, methicillin-resistant staphylococci were described almost as soon as the drug was introduced.[2] After the introduction of methicillin, the number of cases of infection with methicillin-resistant *S aureus* increased greatly in hospitals in Europe, and it became the primary nosocomial pathogen.[3] In the 1960s and even early into the 1970s, the occurrence of methicillin-resistant *S aureus* in US hospitals was rare, but beginning in the 1970s and continuing into the 1990s, the prevalence of methicillin-resistant *S aureus* has increased steadily in US institutions.[1] For many years, these organisms were thought to occur only as pathogens in hospitals, but it is now recognized that they may be found in the community as well. In our recent surveillance studies of institutions throughout the United States, we have found that the overall incidence of methicillin-resistant *S aureus* is 18% (unpublished data), the increase in this organism going from less than 5% in the early 1970s. During the earlier years of increase, methicillin-resistant *S aureus* was shown to be principally a problem in large university-affiliated institutions, but now all hospitals, regardless of size or affiliation, have some level of cases of methicillin-resistant *S aureus* infection.[5]

The staphylococci that are resistant to methicillin and the other penicillinase-resistant penicillins are resistant because they have a unique penicillin-binding protein called PBP2a.[6] The gene for resistance to methicillin is called *mecA* and is incorporated into the chromosome of the *S aureus*. The most important thing about methicillin-resistant *S aureus*, however, is probably not that it is resistant to methicillin, but rather that it is multiply resistant. Many of the methicillin-resistant *S aureus* organisms now identified in the United States in clinical specimens will be resistant to all drugs that are usually tested, except vancomycin. These resistance patterns include resistance to penicillins, cephalosporins, chloramphenicol, the macrolides, lincosamides, tetracyclines (except for minocycline), aminoglycosides, and the fluoroquinolones (Table 2). Only vancomycin is active against all strains of methicillin-resistant *S aureus*. Although these organisms are resistant to most of the drugs that we test, that is not true for methicillin-susceptible *S aureus*. In general, methicillin-susceptible *S aureus* is susceptible to all agents except the β-lactams that are susceptible to hydrolysis by the β-lactamase produced by these organisms.

The coagulase-negative staphylococci, and particularly *Staphylococcus epidermidis*, have long been recognized as being among the more common antibiotic-resistant bacteria that are identified in hospitals. The most resistant species tend to be *S epidermidis*, *Staphylococcus hominis*, and *Staphylococcus haemolyticus*, which also happen to be the most common species found in clinical specimens. The incidence of methicillin resistance in *S epidermidis* has always been much higher than that found for methicillin-resistant *S aureus*. For example, in the 1970s, 15% to 20% of cases of *S epidermidis* were resistant to methicillin. It is not unusual now to find within an institution that 70% to 80% of *S epidermidis* isolates are resistant to methicillin. Although there is some correlation between a resistance to methicillin and that to other agents, it is not nearly as clear or as pronounced as it is in methicillin-resistant *S aureus*. Like methicillin-resistant *S aureus*, most *S epidermidis* isolates produce β-lactamase and are resistant to penicillins; resistance to methicillin is due to the presence of the novel PBP2a within the strains. Because of the high incidence of multiply resistant *S aureus* and *S epidermidis* in hospitals, and because almost

TABLE 1.—*Most Frequent Nosocomial Pathogens When All Anatomic Sites Are Considered**

Rank	Species
1	*Escherichia coli*
2	Enterococci
3	*Pseudomonas aeruginosa*
4	*Staphylococcus aureus*
5	Coagulase-negative staphylococci

*From the National Nosocomial Infections Surveillance studies.[1]

TABLE 2.—*The Usual Antimicrobial Susceptibility and Resistance of Methicillin-Resistant* Staphylococcus aureus *to Antistaphylococcal Agents**

Generally Susceptible to	*Generally Resistant to*
Vancomycin	Penicillins
Teicoplanin†	Cephalosporins
Minocycline‡	Tetracycline
Rifampin§	Macrolides
Sulfamethoxazole-trimethoprim§	Lincosamides
Coumermycin†	Chloramphenicol
Novobiocin sodium‖	Fluoroquinolones
Fusidic acid†	Aminoglycosides

*The overall incidence of methicillin-resistant *S aureus* in the United States is 18%.
†These agents are not available for therapeutic use in the United States.
‡Most isolates are susceptible to minocycline even though resistant to tetracycline.
§Two thirds to three fourths of isolates are generally susceptible to these agents.
‖Novobiocin is rarely used in human medicine.

TABLE 3.—*Screening Tests for High-Level Aminoglycoside Resistance in Enterococci**

Medium	*Antimicrobial Agent*	*Concentration, μg/ml*
Brain-heart infusion broth	Gentamicin	500
	Streptomycin	1,000
Brain-heart infusion agar	Gentamicin	500
	Streptomycin	2,000

*From the National Committee for Clinical Laboratory Standards.[8,9]

all of them are susceptible to vancomycin, vancomycin has become a major antibiotic in terms of the amount used and the dollars spent within US hospitals.

Emerging Resistance in Enterococci

For many years, enterococci have been recognized as being relatively more resistant because they were either resistant or had low-level resistance to many commonly used antimicrobial agents. Despite this, some antimicrobial agents, such as ampicillin, were thought to be active against them. Indeed, for many years there were movements to drop the susceptibility testing of enterococci in clinical laboratories. Enterococcal infections that were not considered to be severe, such as urinary tract infections, were treated with ampicillin. For more serious infections, such as endocarditis due to enterococci, it was considered essential to add a second agent to achieve bactericidal activity and to eradicate the organism, so enterococcal endocarditis was treated with a combination of penicillin and streptomycin that created a synergistic action. It was later discovered that this combination did not always cure patients. The reason was that the enterococcus had developed high-level resistance to streptomycin: synergy between it and penicillin could not be achieved. This high-level resistance was caused by a gene that stimulated the production of an enzyme that inactivated the aminoglycoside. Fortunately, when the organism had high-level resistance to streptomycin, it was susceptible to gentamicin. Thus, gentamicin replaced streptomycin as the aminoglycoside in the combination of penicillin-aminoglycoside therapy for endocarditis. Unfortunately, some enterococci have developed a high-level resistance to gentamicin, resulting in the failure to achieve synergy with a penicillin. Surveillance studies have shown that in the United States, 25% of enterococcal isolates may have resistance to gentamicin.[7] Thus, it is now important to determine possible synergy by testing whether or not the strain of enterococcus shows high-level resistance to the aminoglycoside. The National Committee for Clinical Laboratory Standards (NCCLS), a private, nonprofit organization, has recently published some guidelines for doing these tests (Table 3).[8,9]

It has been shown that strains of enterococci may be resistant to penicillin by two mechanisms. The first is the production of a β-lactamase. The amount of β-lactamase normally produced by an enterococcal strain that is resistant to ampicillin is relatively small. In the usual tests for these strains, resistance may not be evident because there is unlikely to be either an increased minimal inhibitory concentration (MIC) or a smaller zone of inhibition around an ampicillin disk indicating resistance. It is likely that unless a specific test for β-lactamase is performed, such as using nitrocefin, the production of β-lactamase will not be recognized. β-Lactamase-producing enterococci are rare, however, and the likelihood of encountering one in most institutions is not great. The second mechanism for resistance to penicillin in enterococci is the presence of altered penicillin-binding proteins. This is more important than β-lactamase production because it occurs much more often. In fact, the incidence of enterococcal resistance to ampicillin due to altered penicillin-binding proteins is probably near 10% in institutions across the United States.

A third important resistance in enterococci, one that causes great concern among clinicians and microbiologists, is the presence of an inducible vancomycin resistance in enterococci.[10] The threat lies in the source of resistance: a gene that is carried on a plasmid, and the possibility that this vancomycin resistance could be transferred by the plasmid to *S aureus*. This transfer has not yet been demonstrated, and there has yet to be an *S aureus* that is confirmed to be resistant to vancomycin.

The major genes that have been described for resistance in the more clinically important enterococci are the *vanA* and *vanB* genes, but *vanC* genes in *Enterococcus gallinarum* have also been extensively described (Table 4). The MICs for the glycopeptides vancomycin and teicoplanin vary with both *vanA* and *vanB*. Vancomycin resistance due to *vanC* is generally low level. The mechanism for resistance to vancomycin in enterococci involves changes in the membrane of these strains associated with the production of novel proteins.[10] Although many species of enterococci are now recognized, the two major species causing infections in humans are *Enterococcus faecalis* and *Enterococcus faecium*. *Enterococcus faecalis* is by far the most frequent pathogen and probably causes about 90% of the enterococcal infections in humans. The other 10% of infections, for the most part, is due to *E faecium*. This creates a good news, bad news scenario—the bad news is that most of the resistance we see in enterococci occurs in *E faecium*, and the good news is that less than 10% of infections caused by enterococci are due to *E faecium*. The incidence of vancomycin-resistant enterococci is difficult to estimate on a national level because it is a local phenomenon—that is, certain institutions will have clusters with substantial numbers, but most do not.

Development of Resistance in *Streptococcus pneumoniae*

Until recently, good clinical practice considered pneumococci to be susceptible to penicillin and, hence, infections caused by pneumococci would respond to penicillin. Even so, penicillin-resistant pneumococcal isolates had been described in the 1960s in patients in the western Pacific.[11] Penicillin-resistant pneumococci

TABLE 4.—*Enterococcal Species in Which the 3 Major Glycopeptide-Resistant Phenotypes Have Been Observed*

	Glycopeptide-Resistant Phenotype		
Species	vanA	vanB	vanC
Enterococcus faecalis	Yes	Yes	No
Enterococcus faecium	Yes	Yes	No
Enterococcus gallinarum	No	No	Yes

were described in this country in the 1970s, as well as failures of cases of pneumococcal meningitis to respond to penicillin therapy.[12] Nevertheless, the number of these strains in the United States was low, and this low level continued into the 1980s. During the 1980s, a study at the Centers for Disease Control showed that the incidence of pneumococci with elevated MICs to penicillin was about 5% in the United States.[13] During this time, other surveillance studies also showed that the incidence of penicillin resistance in pneumococci in the United States was about 5%.[14] In the early 1990s, however, we did a surveillance study that showed that the incidence of resistance was about 17% to 20%, depending on the methods used to do the susceptibility testing.[15] The development of penicillin resistance in pneumococci is shown in Table 5.

To understand the development of penicillin resistance in pneumococci, one must understand the break points and the definitions that have been used over the years. The NCCLS considers an MIC of 0.06 μg per ml or less as indicating susceptibility; resistance is shown by an MIC of 2 μg per ml or more.[9,16] The in-between MICs of 0.12 to 1 μg per ml were originally described by us as being relatively resistant and are now described by the NCCLS as being intermediate.[16] Although using MIC tests, as described by the NCCLS, is an excellent method for testing pneumococci, this method was not available to most institutions in the United States when resistance in pneumococci was first being studied. Because most laboratories at that time did disk diffusion tests, it was reasonable that penicillin disk diffusion tests were done. These tests worked well if the MICs were high or low—that is, resistant or susceptible—but did not work well if the MICs were within the relatively resistant or intermediate range. It was found, however, that when the oxacillin disk diffusion test was used as a test for penicillin resistance, a much more accurate result was obtained than when using a penicillin disk.[16] This has come to be known as the oxacillin screening test for penicillin resistance in pneumococci. In this test, the 1-μg disk (the same disk used for testing other species) is used for testing pneumococci. A zone of 19 mm or less indicates resistance, and a zone of 20 mm or more indicates susceptibility to penicillin. When these tests were first described, there was total correlation between these disk diffusion break points and the MIC break points described above.

Although the correlation between the oxacillin disk test and the penicillin MIC break points was initially 100%, this has not remained so. Over the years, more and more strains have been isolated that have MICs of 0.06 μg per ml or 0.03 μg per ml (indicating susceptibility) but yet having zone sizes of 19 mm or less, indicating some degree of resistance. In our 1992 studies, the difference was about 3%, but in our 1995 studies, the difference between these two tests was about 10%. In other words, in the 1995 strains, the resistance by the oxacillin screening test was 10% higher than when tested by MICs (in both cases this includes both resistant and intermediate strains). It has been shown that strains that have MICs of 0.06 μg per ml or less but that have zone sizes of 19 mm or less have altered penicillin-binding proteins. They, therefore, have the mechanism for penicillin resistance, although not enough, apparently, to make the strain resistant.

The mechanism of penicillin resistance in pneumococci, as indicated, is the presence of altered penicillin-binding proteins, which have decreased affinity for penicillin (Table 6).[6] These altered penicillin-binding proteins have a decreased affinity not only for penicillin, but also for cephalosporins. In general, this decreased affinity is more evident in the results with oral cephalosporins, but recently increased resistance to third-generation cephalosporins has become more evident.[17] Penicillin-resistant pneumococci also tend to have increased resistance to the macrolides.[18]

Penicillin resistance in pneumococci has developed slowly over the years, but more recently appears to have increased at a faster rate by increasing from about 5% in the latter part of the 1980s to about 20% in 1991-1992. Apparently substantial increases also occurred in 1995.[17] Also of clinical importance is that the incidence of unmistakably penicillin-resistant strains—that is, strains with MICs of 2 μg per ml or more—has almost tripled nationwide (it was about 2.5% in 1992 and now is >6%). If a pneumococcus exhibits intermediate or relative resistance to penicillin and is causing a case of infection other than meningitis, such as upper respiratory tract infection, it is likely that adequate doses of penicillin could be used for cure, although the amount of clinical data that exist to demonstrate this are limited. It is unlikely, however, that anyone would attempt to use a penicillin, a cephalosporin, or a macrolide for the treatment of an infection due to a strain of pneumococcus having an MIC of 2 μg per ml because the chances are it would be resistant in vitro to these agents (see Table 6). Vancomycin would probably be the drug of choice for

TABLE 5.—*The Development of Resistance to Penicillin in* Streptococcus pneumoniae *in the United States*

Time Period	*Incidence, %*
Before 1979	Occasional strains
1979 to 1988*	4-5
1991 to 1992†‡	15.2 (MIC)§; 20.1 (oxacillin screen test)‡

*From Spika et al[13] and Jorgensen et al.[14]
†From Thornsberry et al.[15]
‡See Thornsberry et al for indication of further increases.[17]
§The minimal inhibitory concentration (MIC) and oxacillin screening tests are those recommended by the National Committee for Clinical Laboratory Standards.[8,9]

TABLE 6.—*Correlation of Penicillin Susceptibility in* Streptococcus pneumoniae *and Susceptibility to Other Antipneumococcal Agents*

Penicillin		
Category	*MIC (μg/ml)*	*Correlations*
Susceptible........	≤0.06	Susceptible to most antipneumococcal agents
Intermediate	0.12-1.0	Some isolates will be resistant to cephalosporins (oral and parenteral) and to macrolides
Resistant..........	≥2.0	Nearly all will be resistant to cephalosporins (oral and parenteral, including third-generation); many will be resistant to macrolides

MIC = minimal inhibitory concentration

such cases because no vancomycin-resistant strains of pneumococci have been identified.

In conclusion, in the first half of the 1990s we have seen increases in antimicrobial resistance in strains of gram-positive cocci of medical importance. This includes the multiple resistance to many antimicrobial agents seen in *S aureus* and *S epidermidis* isolates that are resistant to methicillin; the development of resistance to penicillins, aminoglycosides, and vancomycin in enterococci; and the development of penicillin, cephalosporin, and macrolide resistance in pneumococci. Clearly we need to continue surveillance of these organisms on both a national and a worldwide basis to detect any further increases in antimicrobial resistance in these organisms or for the emergence of new resistances.

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